Supplementary methods

Immunohistochemical analysis. Mice kidney tissues were fixed overnight in 4% paraformaldehyde. Paraffin-embedded kidney tissues were cut at a thickness of 4 μm. The sections were incubated with an anti-mATRAP antibody, and detection was achieved using the Histofine Kit (Nichirei Biosciences) and staining with 3,3′-diaminobenzidine.

Supplementary FigureS1

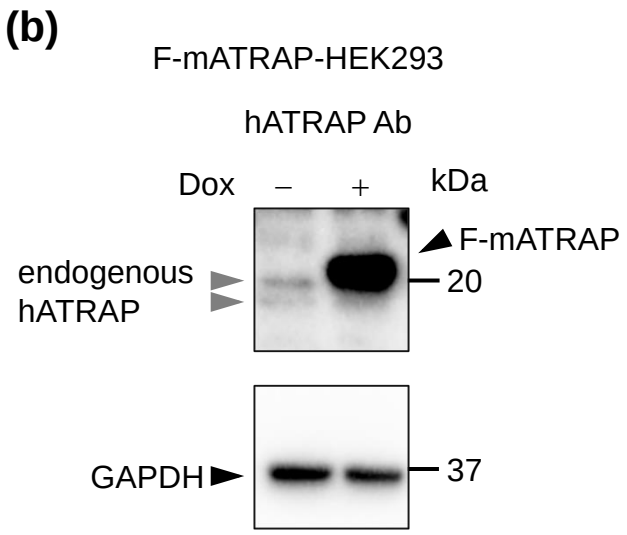
(a)

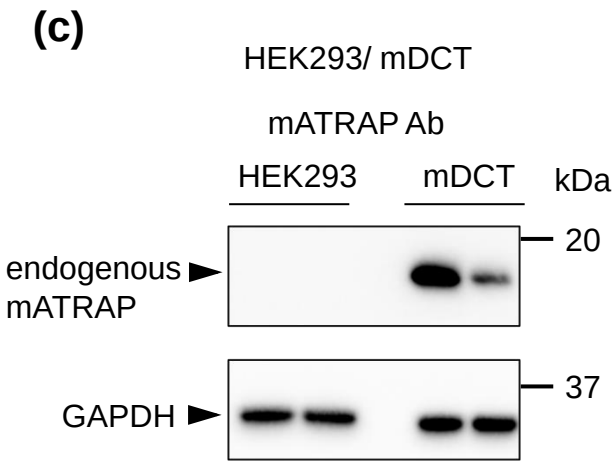
Human ATRAP

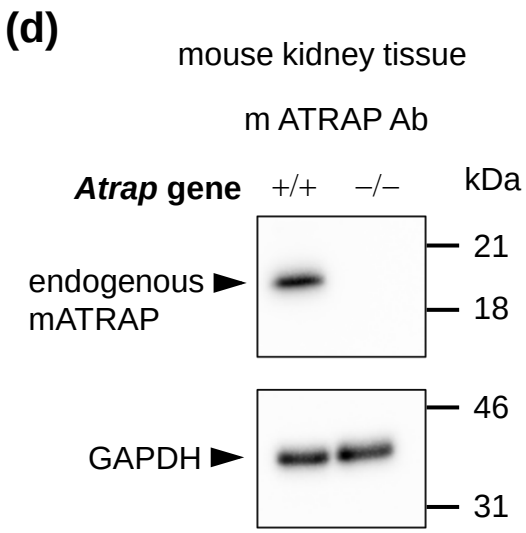
MELPAVNLKV ILLGHWLLTT WGCIVFSGSY AWANFTILAL GVWAVAQRDS IDAISMFLGG **60**
LLATIFLDIV HISIFYPRVS LTDTGRFGVG MAILSLLLKP LSCCFVYHMY RERGGELLVH **120**
TGFLGSSQD RSAYQTIDSA EA**PADPFAVP EGR**SQDARGY **159**

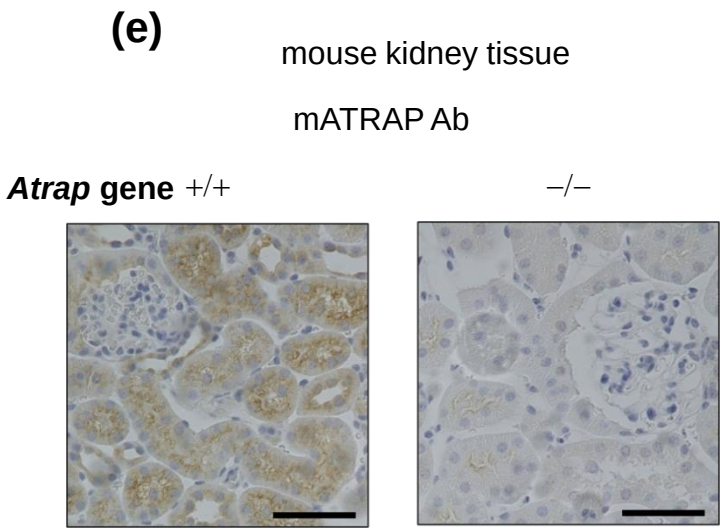
Mouse ATRAP

MELPAVNLKV ILLVHWLLTT WGCLVFSSSY AWGNFTILAL GVWAVAQRDS IDAIGMFLGG **60**
LVATIFLDII YISIFYSSVA TGDTGRFGAG MAILSLLLKP FSCCLVYHMH RERGGELPLR **120**
PDFFGPSQEH SAYQTIDSSS DAAADP**FASL ENKGQAVPRG Y** **161**







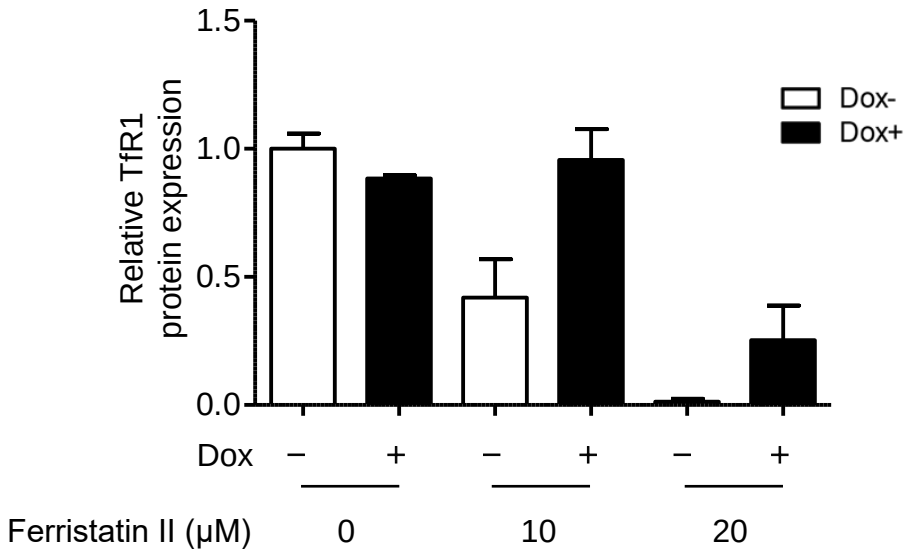


Supplementary FigureS1

Specificity of an anti-mATRAP antibody developed in this study.

(**a**) Amino acid sequences of human and mouse ATRAP. The epitopes for the anti-hATRAP and anti-mATRAP antibodies are indicated with bold and underlined text. (**b**) Total cell lysates from F-mATRAP HEK293 cells, treated with or without Dox (Dox+/-), were analyzed with an anti-hATRAP antibody. The anti-hATRAP antibody detected both endogenous hATRAP and exogenous F-mATRAP. (**c**) Total cell lysates from human (HEK293) and mouse (mDCT) cells were analyzed with an anti-mATRAP antibody. (**d**) Total extracts from mouse kidneys of *ATRAP*-wild type (+/+) and systemic *ATRAP*-KO (-/-) mice were probed with an anti-mATRAP antibody. (**e**) Immunohistochemical analysis of mATRAP expression in kidney tissues from *Atrap*-wild type (+/+) and systemic *Atrap*-KO (-/-) mice. Positive mATRAP staining was evidenced by the presence of brown dots. Scale bars: 50 μm

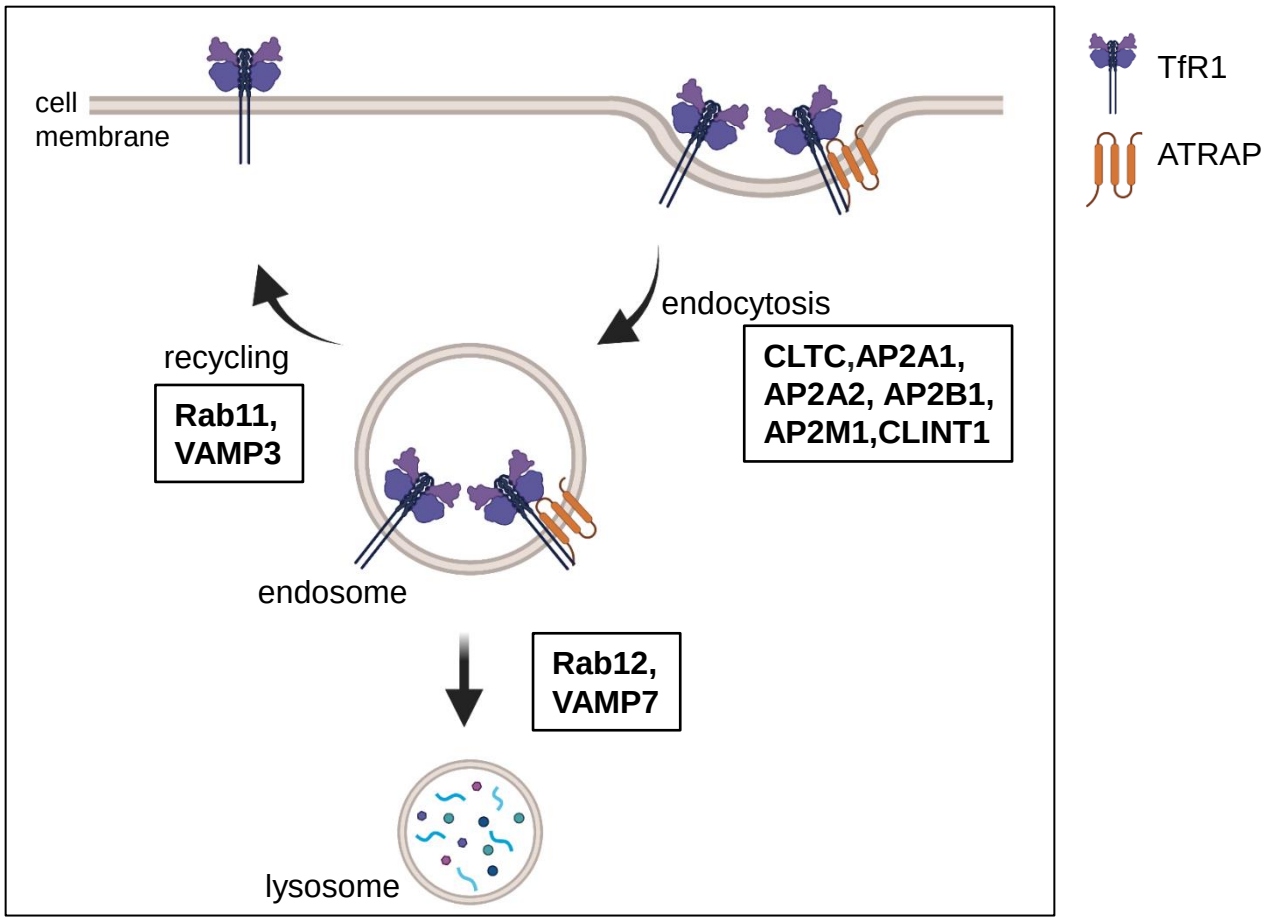
Supplementary FigureS2



Effects of enhanced ATRAP expression on ferristatin II-induced TfR1 degradation.

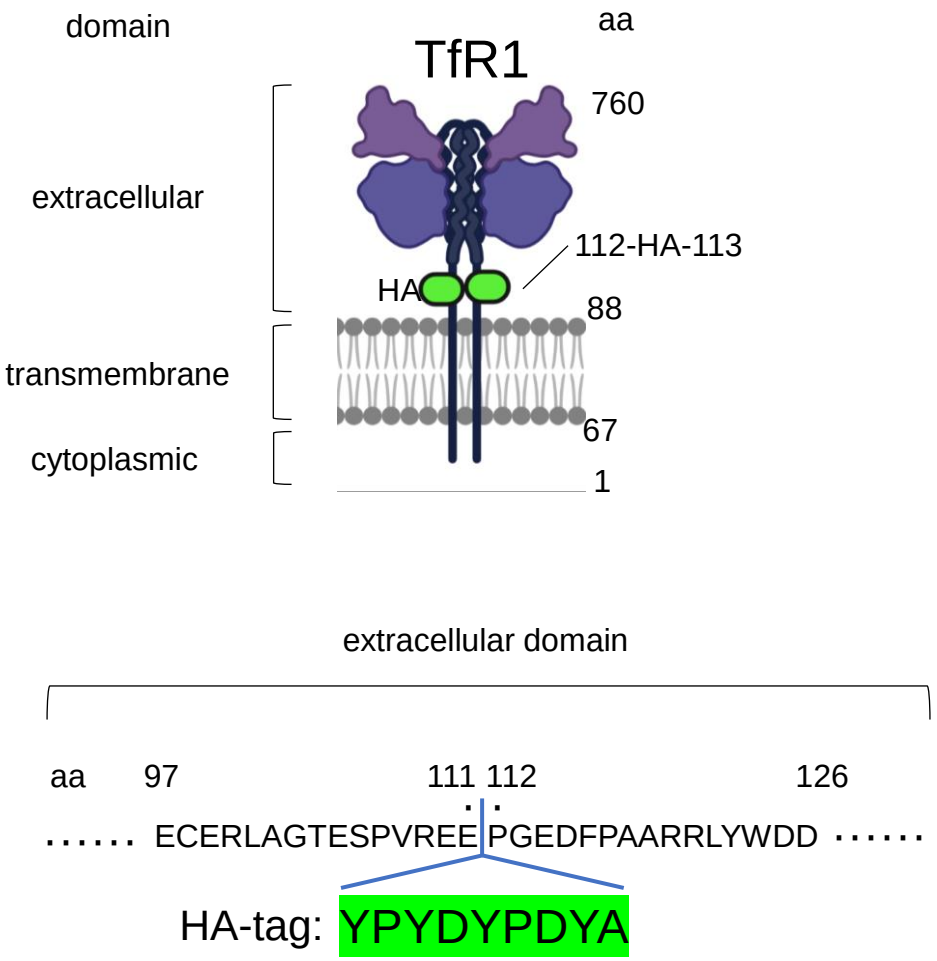
Total cell lysates from F-mATRAP HEK293, treated with or without Dox (Dox+/-), following ferristatin II treatment (0, 10, or 20 μM) for 24 h were probed with an anti-mATRAP antibody. The western blot results for TfR1 protein expression were quantified and plotted (n = 2).

Supplementary FigureS3



Schematic representation of the cellular localization of several candidates of F-mATRAP-associated proteins related to endocytic recycling identified in this study. Clathrin-mediated endocytosis factors: CLTC, AP2A1, AP2A2, AP2B1, AP2M1, and CLINT. Recycling endosome-related factors: Rab11 and VAMP3. Endosome–lysosome pathway-related factors: Rab12 and VAMP7. This figure was created at biorender.com.

Supplementary FigureS4

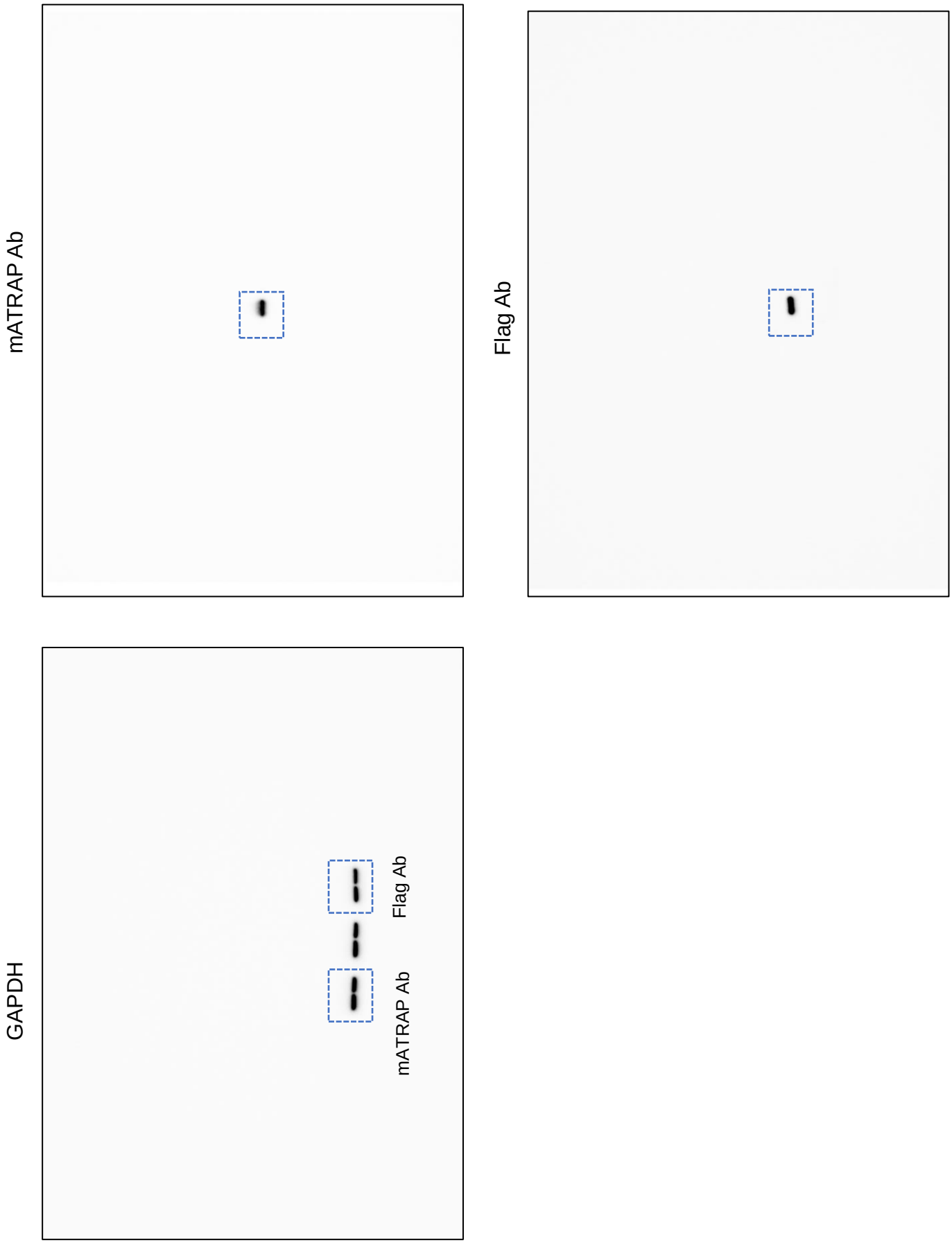


Schema of HA-tagged-TfR1.

Schematic replantation of HA-TfR1 (top) and detailed sequence of HA tag-inserted region (bottom). Amino acid (aa) numbers 1-67 and 88-760 indicate cytoplasmic region and extracellular region of TfR1, respectively. HA-tag was inserted between aa 112 and 113. This figure was created at biorender.com.

supplementary data

A whole gel of Figure1a

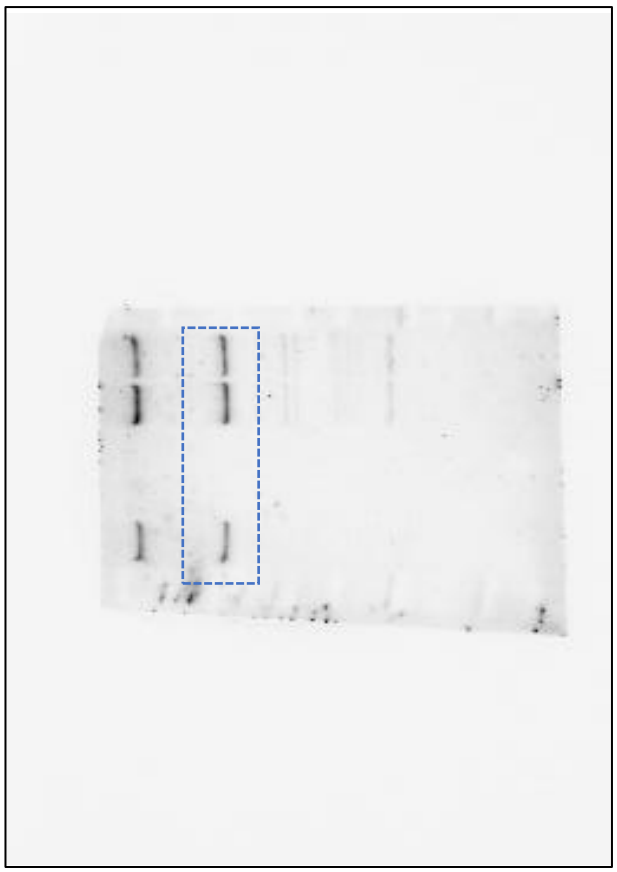


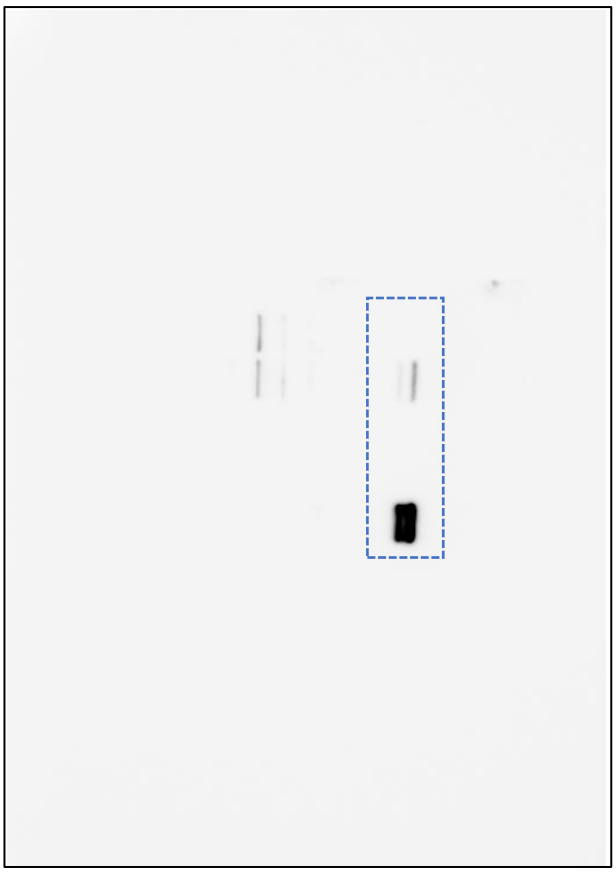
supplementary data

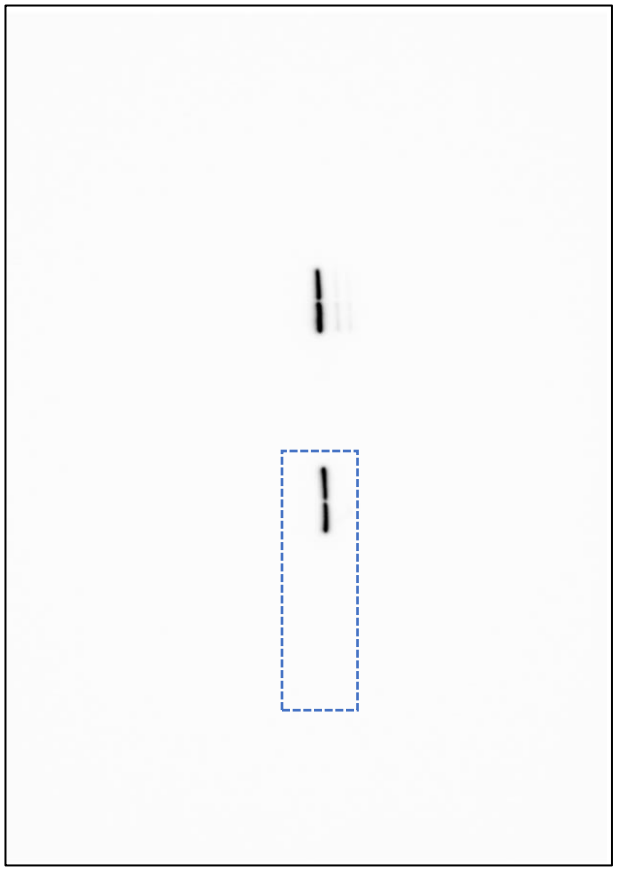
A whole gel of Figure3a

supplementary data

A whole gel of Figure3b



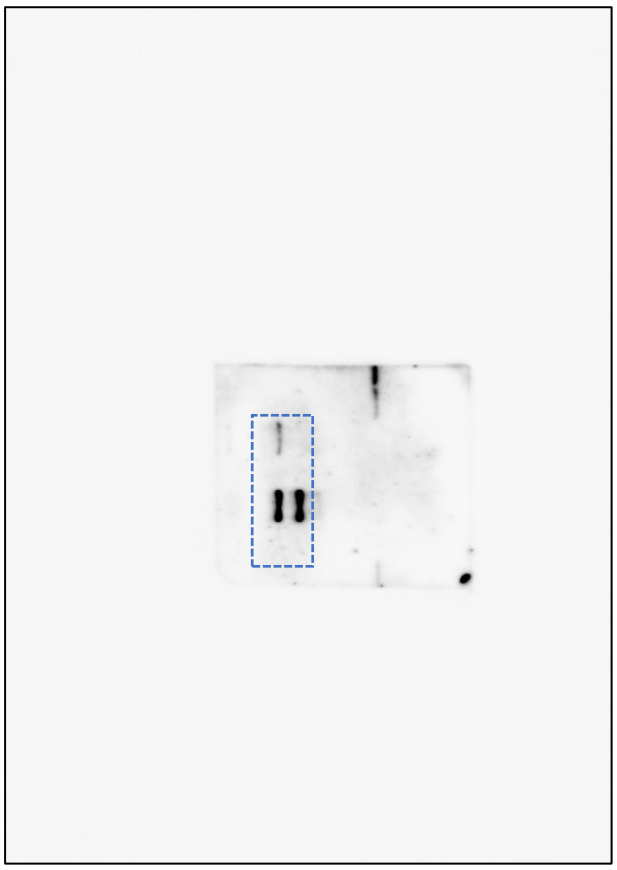




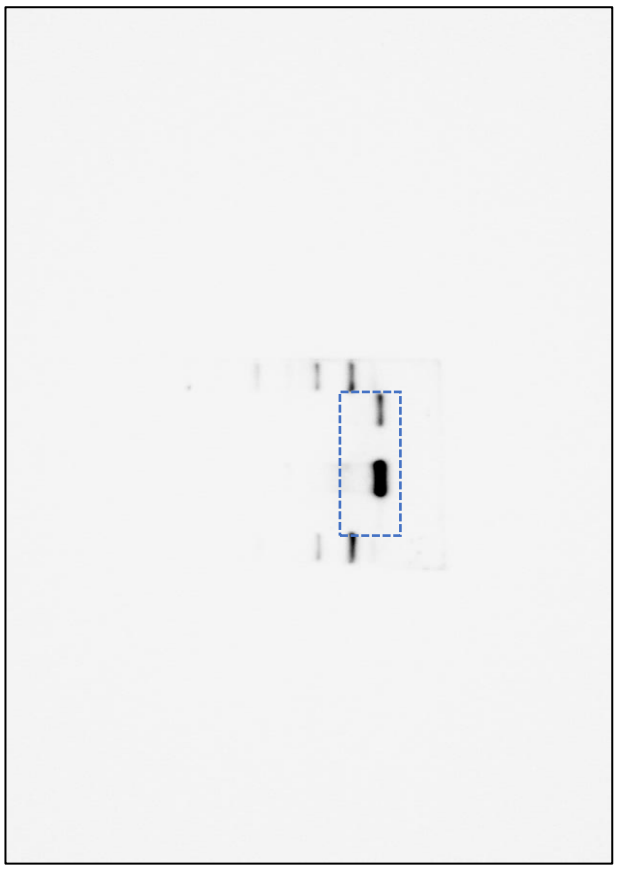
supplementary data

A whole gel of Figure3c

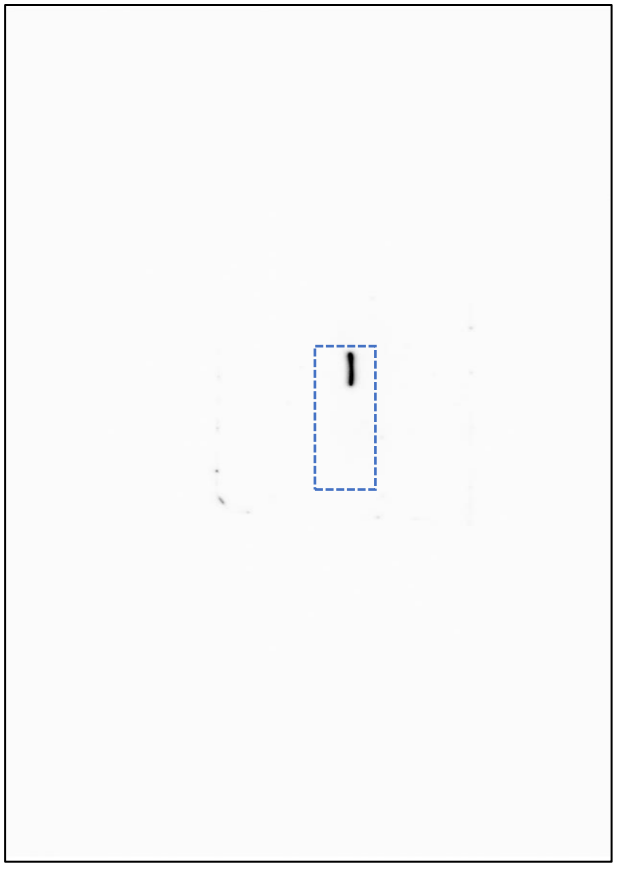
TfR1



F-mATRAP



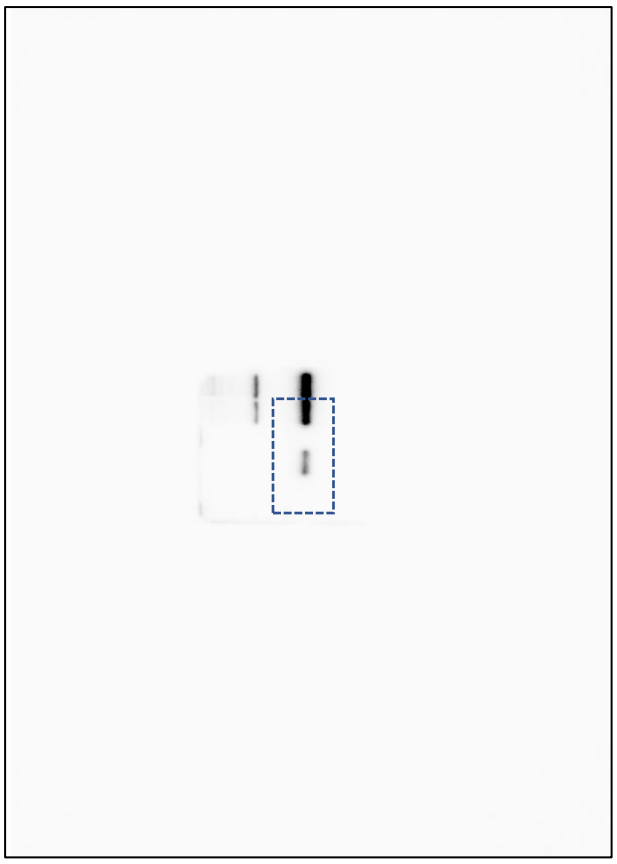
GAPDH



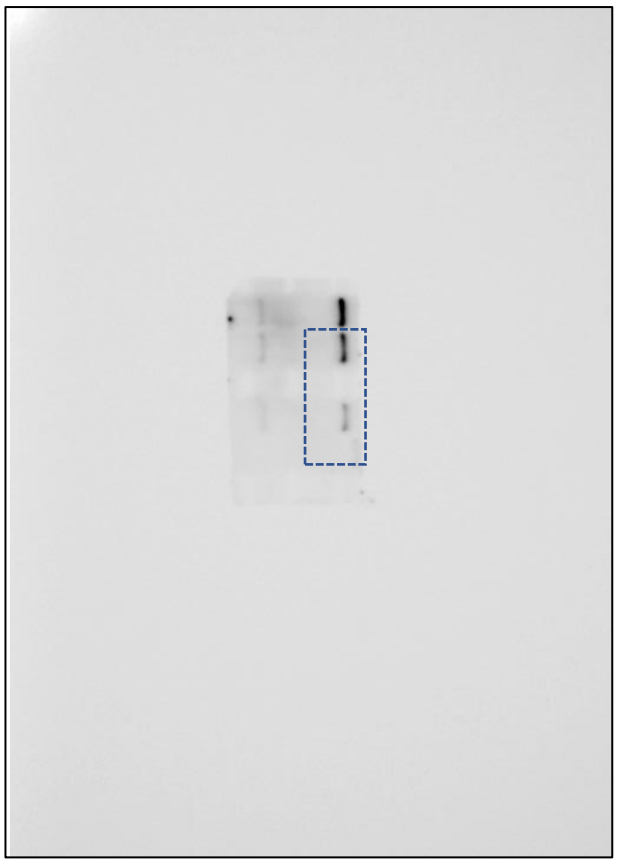
supplementary data

A whole gel of Figure3d

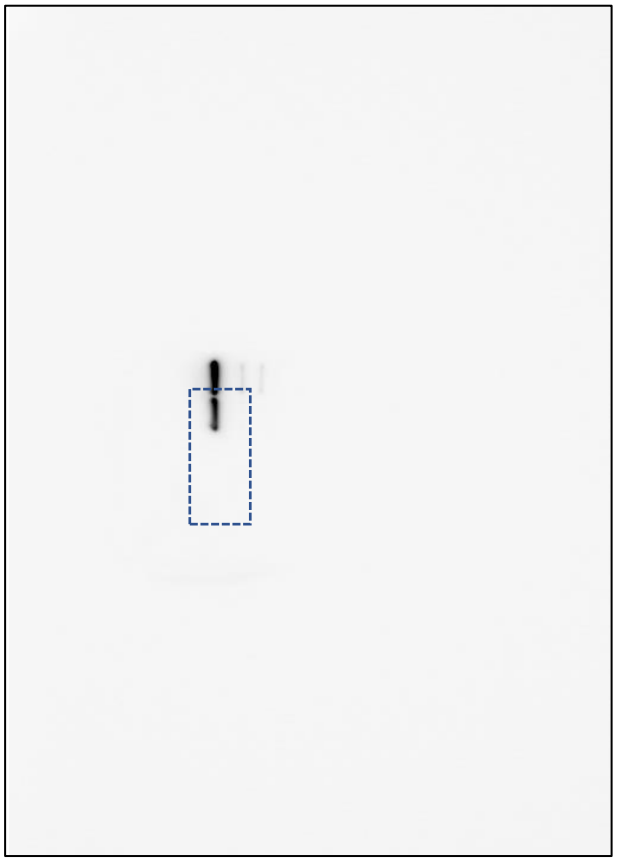
F-mATRAP



TfR1

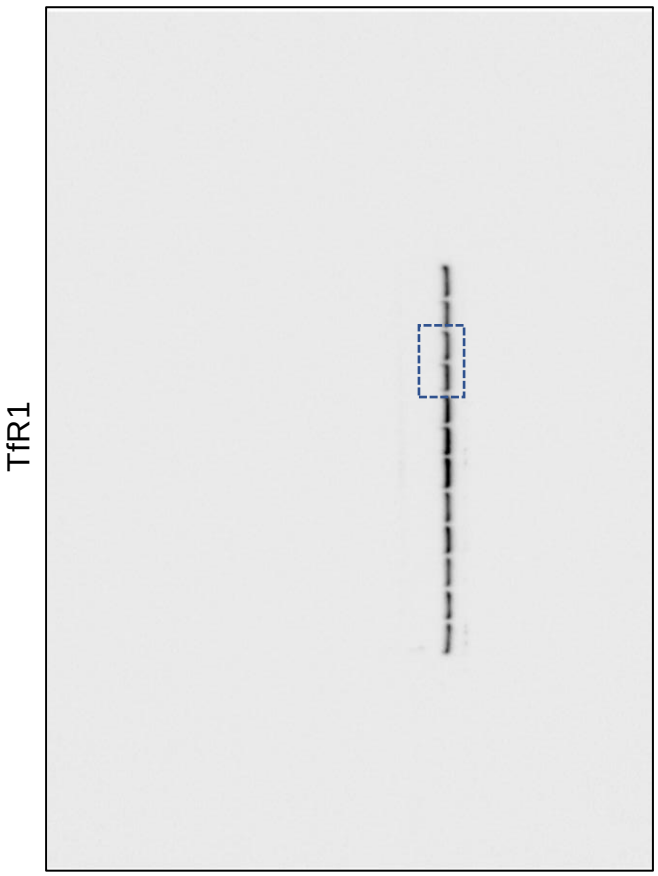


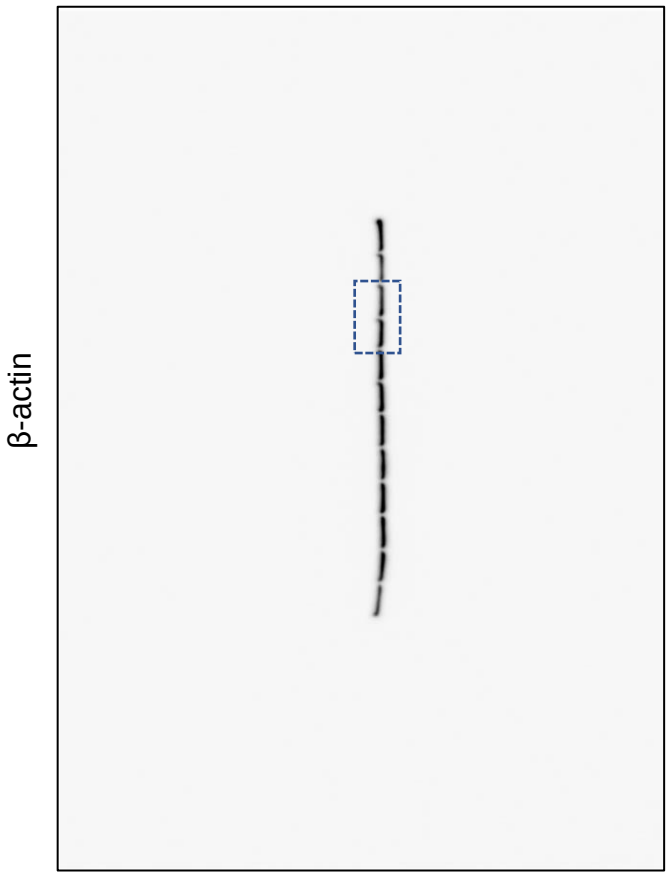
GAPDH

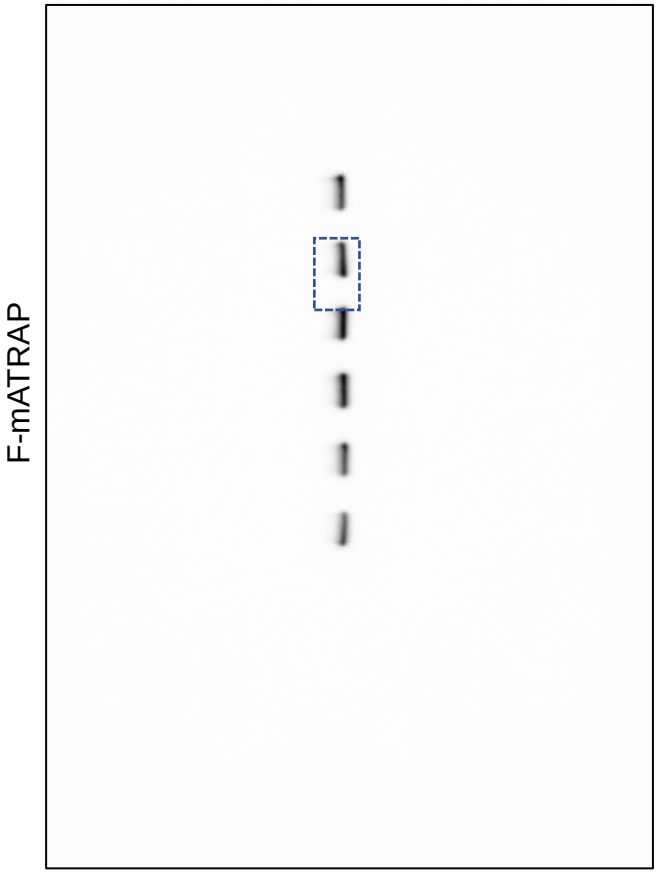


supplementary data

A whole gel of Figure5a

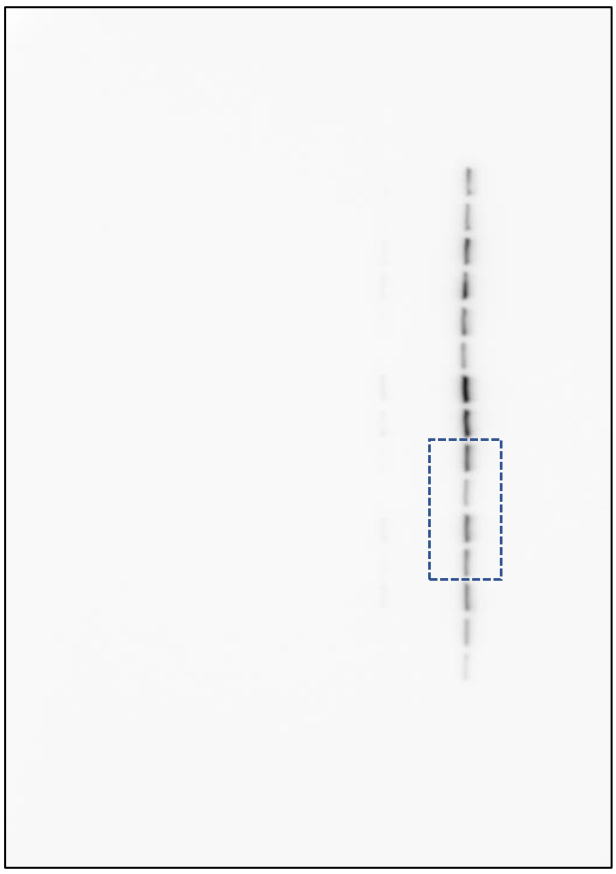


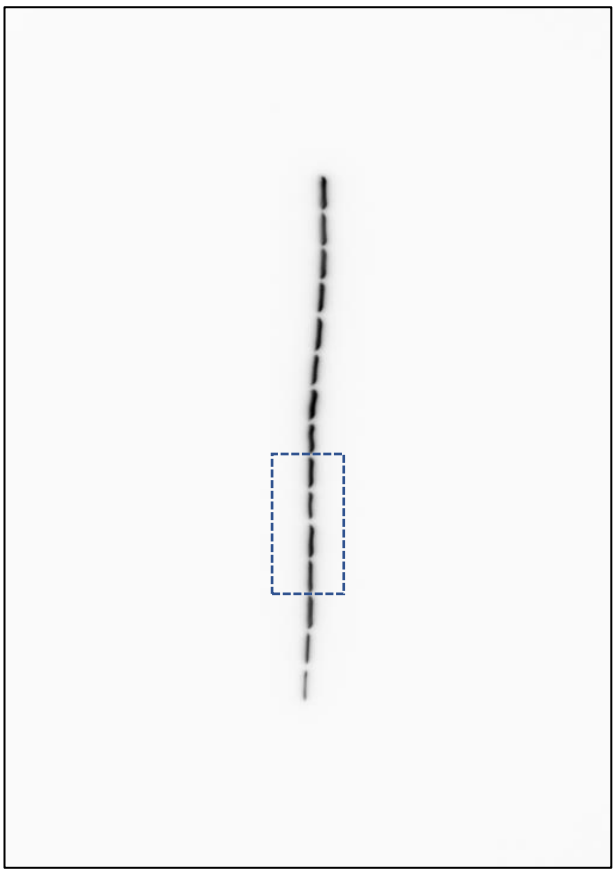


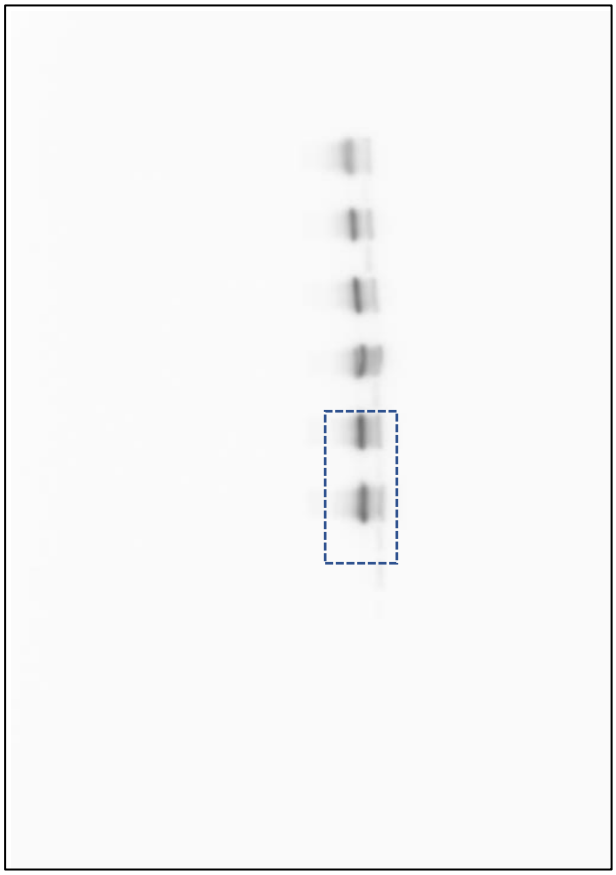


supplementary data

A whole gel of Figure7

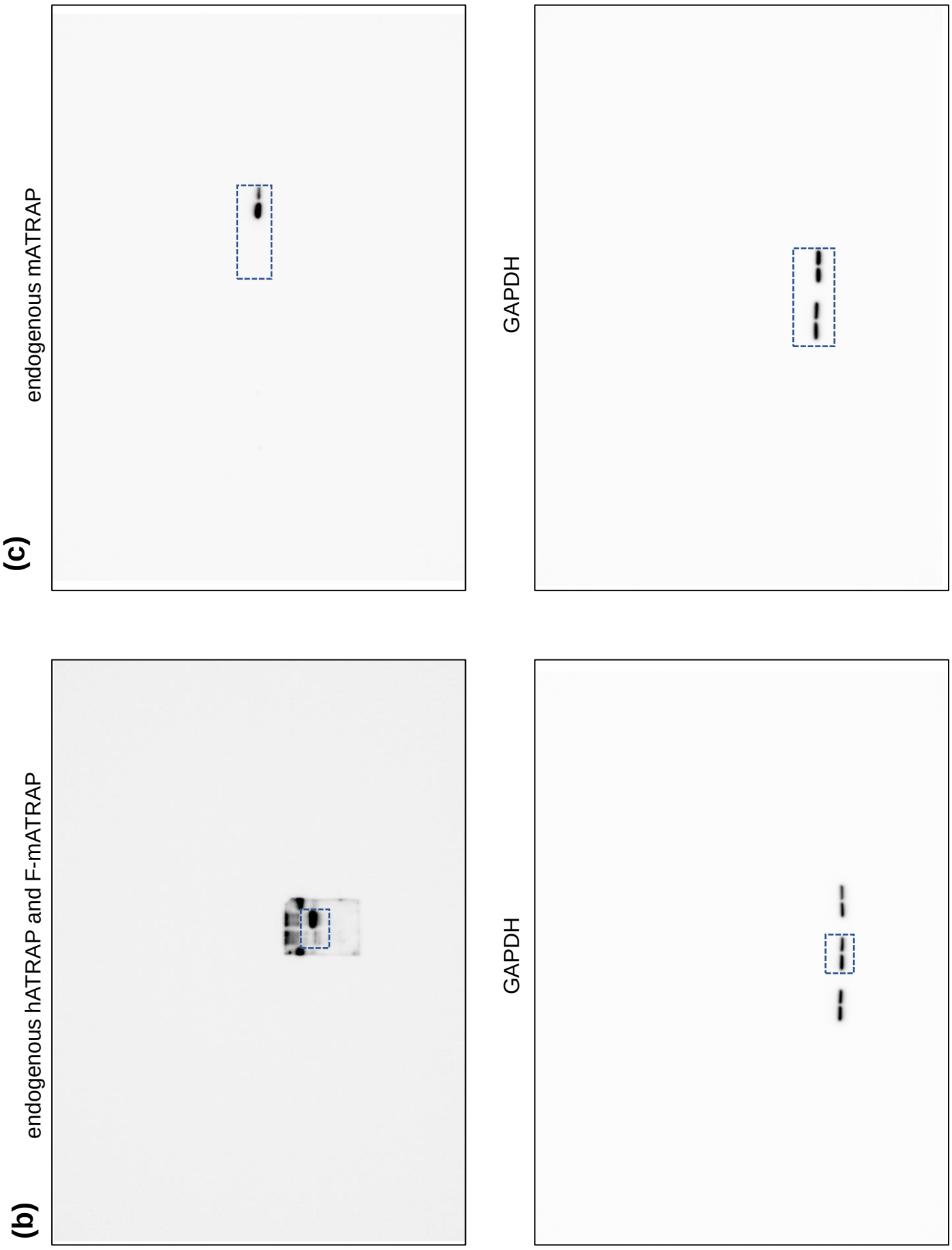






supplementary data

A whole gel of FigureS1



supplementary data

A whole gel of FigureS1

(d)